FEBS Letters 587 (2013) 1008–1011

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Protein dynamics in living cells studied by in-cell NMR spectroscopy

Conggang Li *, Maili Liu

Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Molecular and Atomic Physics, Wuhan Center for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430072, PR China

ARTICLE INFO

Article history:
Received 15 November 2012
Revised 18 December 2012
Accepted 18 December 2012
Available online 11 January 2013

Edited by Wilhelm Just

Keywords:
In-cell NMR
Protein dynamic

ABSTRACT

Most proteins function in cells where protein concentrations can reach 400 g/l. However, most quantitative studies of protein properties are performed in idealized, dilute conditions. Recently developed in-cell NMR techniques can provide protein structure and other biophysical properties inside living cells at atomic resolution. Here we review how protein dynamics, including global and internal motions have been characterized by in-cell NMR, and then discuss the remaining challenges and future directions.

1. Introduction

The cell is the basic unit of life and contains many tightly regulated molecular machines, all of which contain proteins. Dynamics are essential for protein function. To maintain their proper function in cells these proteins must operate at the right place and at the right time. Protein dynamics, including translation and rotation of the entire molecule, domain reorientation, conformation exchange, side chain rotation and bond vibration, occurs on time scales ranging from picoseconds (ps) to second (s)—over 12 orders of magnitude [1–3]. To date, numerous experiments have demonstrated that dynamics are indispensible for all aspects of protein function, including catalysis, ligand binding and allosteric regulation [4–7].

Fluorescence spectroscopy or imaging is the most widely used technique to probe protein dynamics in living cells by tagging fluorescent proteins (FP) to the protein of interest [8]. However, the technique is mostly used to monitor location and the change in location with time [9,10]. Although FRET (Förster resonance energy transfer) can monitor protein conformation changes with high temporal resolution, its currently highest spatial resolution is limited to ~20 nm. Another limitation is that the large size of FP (~27 kDa) can affect the dynamics of the target protein; yet specific labeling of target proteins with small chromophores in living cells remains challenging [11,12].

Nuclear magnetic resonance (NMR) spectroscopy is relatively insensitive compared to fluorescence-based techniques, but can provide dynamic information at atomic resolution. Furthermore, unlike linking FP to the target protein, stable isotope labeling has negligible effects on properties. NMR is a well established technique for probing protein dynamics with a myriad of pulse sequences capable of measuring motion on time scales from ps to s [2,4–6,13,14]. Most such experiments, however, are performed using a single purified protein in dilute solution; conditions vastly different from those in cells in terms of both macromolecule concentrations (up to 300–400 g/l) and redox properties [15–17]. In-cell NMR provides an opportunity to capture intracellular protein dynamics at the atomic level. The critical parameters for in-cell NMR and its application to protein structure, stability, post-translational modification and interactions have been reviewed [18–33]. Here we focus on dynamics, then discuss the challenges and future of in-cell NMR studies aimed at assessing protein dynamics.

2. Global motion in cells

Understanding protein diffusion in cells is essential when the process is rate limiting. Translational and rotational diffusion of proteins of several sizes have been investigated using fluorescence-based techniques. NMR is less sensitivity than fluorescence, requiring mM concentration. The concentration of the most abundant endogenous protein is ~100 μM. The concentration of most proteins in cells is sub-μM, and protein NMR signals from cells are usually broad due to increased viscosity and/or non-specific interactions [20,23,25,27,34]. Moreover, huge background signals from other cellular compounds overlap signals from the target protein, so endogenous proteins in cells are normally invisible, and only abundant small metabolites are detected. The current practice

* Corresponding author. Fax: +86 27 87199291.
E-mail address: conggangli@wipm.ac.cn (C. Li).

0014-5793
http://dx.doi.org/10.1016/j.febslet.2012.12.023

for in-cell NMR is to selectivity isotopically enrich (or label) the target proteins with NMR-sensitive nuclei so as to obtain distinguishable signals. In addition, the target protein is generally over-expressed (up to mM), which may negative the biological relevance of the results. Nevertheless, some abundant endogenous metalloproteins contain paramagnetic metal ions that induce large chemical-shift dispersion, resulting in characteristic NMR signals separated from other diamagnetic proteins. Histidyl $N_\delta H$ and the Val E11 ΥCH_3 of myoglobin in cells or tissues are visible [35].

Protein dynamics are reflected in the longitudinal relaxation rate, R_1, and transverse relaxation rate, R_2, of the observed nuclei. Both rates are sensitive to global motion. Increased viscosity or interaction with other macromolecules leads to small R_1 values and large R_2 values. If R_1 is too small, the magnetization relaxes slowly between pulses, lowering sensitivity. If the R_2 is too large, the resonances are too broad to observe. In a paramagnetic molecule, R_2 depends on the square of magnetic field strength. By analyzing the field-dependence of R_2, myoglobin and hemoglobin were found rotationally diffuse 1.4- and 2.2-times more slowly in perfused myocardium than they do in dilute solution [35].

Other strategies to distinguish target protein signals from background include specifically isotope labeling and target protein over-expression. Hemoglobin specifically ^{13}C labeled at the C-2 histidine position was used to measure intracellular viscosity in erythrocytes. By comparing the longitudinal relaxation time T_1 before and after hemolysis, London et al. found that myoglobin rotates about two times more slowly in erythrocytes than it does in water [36]. ^{15}N enriched protein GB1 domain (GB1, 6 kDa) over expressed in *Escherichia coli* shows resolved cross peaks in two-dimensional HSQC spectra. Resonance line width is proportional to global motion and in principal can be used to measure rotational diffusion. Unfortunately, conformation exchange, sample inhomogeneity and non-specific interactions also contribute. However, the difference in widths between ^{15}N transverse relaxation optimized spectra (TROSY) and anti-TROSY spectra, $\Delta\Delta\upsilon$TAT is independent of chemical exchange and sample inhomogeneity. The Gierasch group found that $\Delta\Delta\upsilon$TAT for purified GB1 depends linearly on the viscosity of glycerol solutions. They used this relationship to show that the rotational diffusion of GB1 is $\sim 8 \pm 2$ times slower in *E. coli* cells than it is in water [20]. This result is consistent with the observation that the translational diffusion of green fluorescence protein in *E. coli* (measured by fluorescence recovery after photo bleaching) is slowed ~11-fold [37].

^{19}F NMR has also been used to study the diffusion of three fluorine labeled proteins, hexokinase, phosphoglycerate kinase, and pyruvate kinase, in yeast cells. Comparison of the ^{19}F longitudinal relaxation times in living cells and in viscogen solutions indicate that phosphoglycerate kinase and hexokinase tumble two times slower in yeast cytoplasm than in dilute solution. Signals from pyruvate kinase in yeast cells were not even detected, indicating some degree motional restriction [38]. ^{19}F NMR has also been employed to study over-expressed proteins in *E. coli*, but quantitative diffusion measurement were not made [39].

The methods discussed above were used to determine global protein motion and were based on comparing relaxation rates in cells to those in viscogen solutions. The approach assumes that only global motion affects relaxation and that internal motion is the same in the cells and in dilute solution. This assumption requires validation. If internal dynamics are different in cells and in solutions, global rotational diffusion will be underestimated or overestimated, depending on how internal dynamics change. It is known that intermolecular interactions affect R_1 and R_2 differently, so apparent rotational diffusion measured from R_1 and R_2 will have different values. Interpretation of global motion from R_2 data is more subject to error because R_2 is more sensitive to conformational exchange, sample inhomogeneity and protein weak interactions, which are ubiquitous in cells [38].

Unlike globular proteins, the global motion of intrinsically disordered protein, is difficult to determine, because their relaxation times shows only weak dependence on global motion and is instead dominated by local internal motions [40,41]. NMR methods to characterize the global motion of intrinsically disordered protein in cells need to be developed.

3. Internal motion in cells

Proteins also undergo local motion that arises from internal dynamics. These motions occur over a wide range of timescales, e.g. side chain movement occurs in ps to ns, loop motion in ns to μs, and domain or subunit motion in μs to ms. These motions have characterized via their effects on relaxation rate (R_1, R_2), heteronuclear NOE (hetNOE), relaxation dispersion, paramagnetic relaxation enhancement (PRE) and residual dipolar coupling (RDC), respectively in dilute solutions [2,3,6,7]. However, protein dynamics have not been measured in living cells, partly because few proteins give high resolution in-cell NMR spectra. Also, such relaxation data can be "contaminated" by transient weak interactions, complicating interpretation, which are ubiquitous in cells. The so-called model free approach for quantifying motional amplitude (i.e., the order parameter, S^2) and timescale (ps to ns) [13,14,42] cannot be simply applied to protein in cells.

Order parameters and their time scales can be overestimated due to the presence of protein weak interactions. Li et al. have developed a method to use product of ^{15}N R_1 and R_2 to estimate weak interactions between target protein and crowding agents under crowding conditions [43,44]. This method can also been applied to in-cell studies. ^{15}N R_1 and R_2 values for several lysines have been measured in lysine-enriched TTHA1718 in *E.coli* cells [24]. The average value of R_1R_2 for these residues are $27.3S^{-2}$, which exceeds the maximum rigid limit line value of about $19.6S^{-2}$ at 600 MHz, suggesting minimal interactions between TTHA1718 and endogenous proteins.

Although it is difficult to obtain internal dynamics data in cells, in vitro experiments that mimic the cellular environment using cell lysates or high concentration polymer solutions have been performed. Miklos et al. found that fast dynamics of amides in chymotrypsin inhibitor 2 (CI2) did not change in solutions crowding with poly(*N*-isopropylacrylamide-co-acrylic acid), where the interaction between CI2 and crowding agent is minimal [45]. Using ^2H relaxation, Latham et al. reported that the fast methyl side chain dynamics of calmodulin (CaM) in 100 g/l cell lysates are very similar to those in dilute solutions [46]. However, it must be borne in mind that for such experiments, there is a negligible contribution from weak interactions because of the dominance of the quadrupolar interaction. These authors also used relaxation dispersion experiments to measure motions on the ms time scale and reported significant processes in apo-CaM and Ca^{2+} bound CaM in lysates that are absent in buffer alone. These ms dynamics are the result of sampling of a metal bound intermediate, not from the interactions with endogenous *E. coli* proteins [47].

4. Perspectives

The dynamic nature of proteins is essential for their function, and protein function depends on environment. Thus, how the cellular environment affects these dynamics is a major concern. Can mimicking the crowded cellular environment provide biologically-relevant information? Are the internal dynamics the same in cells and buffer? Answering these questions requires new experimental techniques and methods.

In-cell NMR is an ideal tool for gaining information about protein dynamics at the atomic level, but several obstacles remain. First, few proteins provide high quality NMR spectra inside cells. Second, in-cell NMR is limited by the life span of the cells in the NMR tube, which can be much less than the time required to acquire high quality relaxation data. Even if cells do not lyse, then may leak the target protein into the media during the NMR experiment, producing artifacts [48,49]. Third, the relaxation data can be "contaminated" by protein interactions, complicating interpretation. Fourth, PRE and RDC experiments, requiring specific paramagnetic labeling and molecular alignment, are difficult to implement in living cells. To overcome these limitations, high sensitivity and high resolution in-cell spectra must be obtained and "interaction free" methods must be developed. In addition, site specific labeling, high fields and cryogenic probes, fast acquisition schemes and new data process methods need to be combined to enhance sensitivity and resolution. Perhaps most importantly, these new methods must be insensitive to weak interactions.

Size limitation will always be present in solution NMR because line widths and internal dynamics are coupled to the total correlation time. Solid-state magic-angle spinning (MAS) NMR does not have a correlation time problem. Instead, the method requires immobility. Recently, several groups have shown that the ultracentrifugation accompanying fast MAS can immobilize soluble proteins at the rotor wall. High quality solid-state like NMR spectra of transiently sedimented molecules can be obtained, overcoming the size limitations of solution NMR [50,51]. In-cell solid-state MAS NMR may therefore be a useful tool to study the structure and dynamics of soluble large protein complexes in cells [23]. Another advance is the recently shown feasibility of using solid-state MAS NMR for detecting membrane proteins in native cell membranes [22,52–54] and living *E. coli* cells [18]. Background signals can be suppressed by advanced isotope labeling strategies and more elaborate multi-dimensional experiments. Dynamic nuclear polarization has also been applied successfully to improve sensitivity in studies of membrane proteins in native cellular membranes [54]. Compared to solution NMR, the most attractive aspect of using solid-state NMR to probe protein dynamics is that overall tumbling is absent and protein internal dynamics can be derived from longitudinal relaxation, dipolar coupling constants and chemical shift anisotropy (CSA) line shape [55]. These advances raise our optimism that it will be possible to study protein dynamics in natural environments.

5. Conclusions

The ultimate goal for protein science is to study proteins in their natural cellular environment in the post-reductionist era of biochemistry. Although many advances have been made for in-cell NMR, studies of protein dynamics are limited. We believe in-cell NMR is an ideal technique for studying protein dynamics at atomic resolution. Nevertheless, concerns, challenges and limitations remain. Developments in instrumentation, isotope labeling, pulse sequences, fast acquisition schemes and data process method will enable protein dynamics studies in cells and expand the application of in-cell NMR.

Acknowledgments

This work was supported by the Ministry of Science and Technology of China (2009CB918600 to M. Liu, 2013CB910200 to C. Li), 1000 plan for young talents program of China and the National Natural Sciences Foundation of China (21075134 and 21173258 to C. Li). We thank Prof. Gary J. Pielak for helpful comments on the manuscript.

References

[1] Henzler-Wildman, K. and Kern, D. (2007) Dynamic personalities of proteins. Nat. Struct. Mol. Biol. 450, 964–972.
[2] Palmer IIIrd, A.G. and Massi, F. (2006) Characterization of the dynamics of biomacromolecules using rotating-frame spin relaxation NMR spectroscopy. Chem. Rev. 106, 1700–1719.
[3] Jarymowycz, V.A. and Stone, M.J. (2006) Fast time scale dynamics of protein backbones: NMR relaxation methods, applications, and functional consequences. Chem. Rev. 106, 1624–1671.
[4] Bouvignies, G. et al. (2011) Solution structure of a minor and transiently formed state of a T4 lysozyme mutant. Nat. Struct. Mol. Biol. 477, 111–114.
[5] Bhabha, G., Lee, J., Ekiert, D.C., Gam, J., Wilson, I.A., Dyson, H.J., Benkovic, S.J. and Wright, P.E. (2011) A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis. Science 332, 234–238.
[6] Mittermaier, A. and Kay, L.E. (2006) New tools provide new insights in NMR studies of protein dynamics. Science 312, 224–228.
[7] Iwahara, J. and Clore, G.M. (2006) Detecting transient intermediates in macromolecular binding by paramagnetic NMR. Nat. Struct. Mol. Biol. 440, 1227–1230.
[8] Lippincott-Schwartz, J., Altan-Bonnet, N. and Patterson, G.H. (2003) Photobleaching and photoactivation: following protein dynamics in living cells. Nat. Cell Biol. (Suppl) S7–S14.
[9] Misteli, T. and Spector, D.L. (1997) Applications of the green fluorescent protein in cell biology and biotechnology. Nat. Biotechnol. 15, 961–964.
[10] Gerdes, H.H. and Kaether, C. (1996) Green fluorescent protein: applications in cell biology. FEBS Lett. 389, 44–47.
[11] Dix, J.A. and Verkman, A.S. (2008) Crowding effects on diffusion in solutions and cells. Annu. Rev. Biophys. 37, 247–263.
[12] Xie, X.S., Choi, P.J., Li, G.W., Lee, N.K. and Lia, G. (2008) Single-molecule approach to molecular biology in living bacterial cells. Annu. Rev. Biophys. 37, 417–444.
[13] Lipari, G. and Szabo, A. (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules. Analysis of experimental results. J. Am. Chem. Soc. 104, 4559–4570.
[14] Lipari, G. and Szabo, A. (1982) Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules. 1. Theory and range of validity. J. Am. Chem. Soc. 104, 4546–4559.
[15] Zimmerman, S.B. and Trach, S.O. (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. J. Mol. Biol. 222, 599–620.
[16] McGuffee, S.R. and Elcock, A.H. (2010) Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm. PLoS Comput. Biol. 6, e1000694.
[17] Zhou, H.X., Rivas, G. and Minton, A.P. (2008) Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. Annu. Rev. Biophys. 37, 375–397.
[18] Renault, M., Tommassen-van Boxtel, R., Bos, M.P., Post, J.A., Tommassen, J. and Baldus, M. (2012) Cellular solid-state nuclear magnetic resonance spectroscopy. Proc. Natl. Acad. Sci. USA 109, 4863–4868.
[19] Reckel, S., Lopez, J.J., Lohr, F., Glaubitz, C. and Dötsch, V. (2012) In-cell solid-state NMR as a tool to study proteins in large complexes. ChemBioChem 13, 534–537.
[20] Wang, Q., Zhuravleva, A. and Gierasch, L.M. (2011) Exploring weak, transient protein–protein interactions in crowded in vivo environments by in-cell nuclear magnetic resonance spectroscopy. Biochemistry 50, 9225–9236.
[21] Maldonado, A.Y., Burz, D.S. and Shekhtman, A. (2011) In-cell NMR spectroscopy. Prog. Nucl. Magn. Reson. Spectrosc. 59, 197–212.
[22] Fu, R., Wang, X., Li, C., Santiago-Miranda, A.N., Pielak, G.J. and Tian, F. (2011) In situ structural characterization of a recombinant protein in native *Escherichia coli* membranes with solid-state magic-angle-spinning NMR. J. Am. Chem. Soc. 133, 12370–12373.
[23] Crowley, P.B., Chow, E. and Papkovskaia, T. (2011) Protein interactions in the *Escherichia coli* cytosol: an impediment to in-cell NMR spectroscopy. ChemBioChem 12, 1043–1048.
[24] Sakakibara, D. et al. (2009) Protein structure determination in living cells by in-cell NMR spectroscopy. Nat. Struct. Mol. Biol. 458, 102–105.
[25] Pielak, G.J., Li, C., Miklos, A.C., Schlesinger, A.P., Slade, K.M., Wang, G.F. and Zigoneanu, I.G. (2009) Protein nuclear magnetic resonance under physiological conditions. Biochemistry 48, 226–234.
[26] Inomata, K. et al. (2009) High-resolution multi-dimensional NMR spectroscopy of proteins in human cells. Nat. Struct. Mol. Biol. 458, 106–109.
[27] Augustus, A.M., Reardon, P.N. and Spicer, L.D. (2009) MetJ repressor interactions with DNA probed by in-cell NMR. Proc. Natl. Acad. Sci. USA 106, 5065–5069.
[28] Banci, L., Barbieri, L., Bertini, I., Cantini, F. and Luchinat, E. (2011) In-cell NMR in *E. coli* to monitor maturation steps of hSOD1. PLoS One 6, e23561.
[29] Selenko, P., Frueh, D.P., Elsaesser, S.J., Haas, W., Gygi, S.P. and Wagner, G. (2008) In situ observation of protein phosphorylation by high-resolution NMR spectroscopy. Nat. Struct. Mol. Biol. 15, 321–329.
[30] Serber, Z. and Dötsch, V. (2001) In-cell NMR spectroscopy. Biochemistry 40, 14317–14323.
[31] Serber, Z., Corsini, L., Durst, F. and Dötsch, V. (2005) In-cell NMR spectroscopy. Methods Enzymol. 394, 17–41.

[32] Selenko, P., Serber, Z., Gadea, B., Ruderman, J. and Wagner, G. (2006) Quantitative NMR analysis of the protein G B1 domain in *Xenopus laevis* egg extracts and intact oocytes. Proc. Natl. Acad. Sci. USA 103, 11904–11909.
[33] Wang, Y., Li, C. and Pielak, G.J. (2012) In-cell protein magnetic resonance spectroscopy. Chin. J. Magn. Reson. 29, 475–488.
[34] Barnes, C.O., Monteith, W.B. and Pielak, G.J. (2011) Internal and global protein motion assessed with a fusion construct and in-cell NMR spectroscopy. ChemBioChem 12, 390–391.
[35] Wang, D., Kreutzer, U., Chung, Y. and Jue, T. (1997) Myoglobin and hemoglobin rotational diffusion in the cell. Biophys. J. 73, 2764–2770.
[36] London, R.E., Gregg, C.T. and Matwiyoff, N.A. (1975) Nuclear magnetic resonance of rotational mobility of mouse hemoglobin labeled with (2–13C)histidine. Science 188, 266–268.
[37] Elowitz, M.B., Surette, M.G., Wolf, P.E., Stock, J.B. and Leibler, S. (1999) Protein mobility in the cytoplasm of *Escherichia coli*. J. Bacteriol. 181, 197–203.
[38] Williams, S.P., Haggie, P.M. and Brindle, K.M. (1997) ^{19}F NMR measurements of the rotational mobility of proteins in vivo. Biophys. J. 72, 490–498.
[39] Li, C. et al. (2010) Protein (19)F NMR in *Escherichia coli*. J. Am. Chem. Soc. 132, 321–327.
[40] Li, C., Charlton, L.M., Lakkavaram, A., Seagle, C., Wang, G., Young, G.B., Macdonald, J.M. and Pielak, G.J. (2008) Differential dynamical effects of macromolecular crowding on an intrinsically disordered protein and a globular protein: implications for in-cell NMR spectroscopy. J. Am. Chem. Soc. 130, 6310–6311.
[41] Wang, Y., Benton, L.A., Singh, V. and Pielak, G.J. (2012) Disordered protein diffusion under crowded conditions. J. Phys. Chem. Lett. 3, 2703–2706.
[42] Schurr, J.M., Babcock, H.P. and Fujimoto, B.S. (1994) A test of the model-free formulas. Effects of anisotropic rotational diffusion and dimerization. J. Magn. Reson. B 105, 211–224.
[43] Li, C., Wang, Y. and Pielak, G.J. (2009) Translational and rotational diffusion of a small globular protein under crowded conditions. J. Phys. Chem. B 113, 13390–13392.
[44] Li, C. and Pielak, G.J. (2009) Using NMR to distinguish viscosity effects from nonspecific protein binding under crowded conditions. J. Am. Chem. Soc. 131, 1368–1369.
[45] Miklos, A.C., Li, C., Sorrell, C.D., Lyon, L.A. and Pielak, G.J. (2011) An upper limit for macromolecular crowding effects. BMC Biophys. 4, 13.
[46] Latham, M.P. and Kay, L.E. (2012) Is buffer a good proxy for a crowded cell-like environment? A comparative NMR study of calmodulin side-chain dynamics in buffer and *E. coli* lysate. PLoS One 7, e48226.
[47] Velyvis, A., Ruschak, A.M. and Kay, L.E. (2012) An economical method for production of (2)H, (13)CH_3–threonine for solution NMR studies of large protein complexes: application to the 670 kDa proteasome. PLoS One 7, e43725.
[48] Barnes, C.O. and Pielak, G.J. (2011) In-cell protein NMR and protein leakage. Proteins 79, 347–351.
[49] Sakai, T. et al. (2007) Fluoroscopic assessment of protein leakage during *Xenopus oocytes* in-cell NMR experiment by co-injected EGFP. Anal. Biochem. 371, 247–249.
[50] Mainz, A., Jehle, S., van Rossum, B.J., Oschkinat, H. and Reif, B. (2009) Large protein complexes with extreme rotational correlation times investigated in solution by magic-angle-spinning NMR spectroscopy. J. Am. Chem. Soc. 131, 15968–15969.
[51] Bertini, I., Luchinat, C., Parigi, G., Ravera, E., Reif, B. and Turano, P. (2011) Solid-state NMR of proteins sedimented by ultracentrifugation. Proc. Natl. Acad. Sci. USA 108, 10396–10399.
[52] Miao, Y. et al. (2012) M2 proton channel structural validation from full-length protein samples in synthetic bilayers and *E. coli* membranes. Angew. Chem., Int. Ed. Engl. 51, 8383–8386.
[53] Kulminskaya, N.V., Pedersen, M.O., Bjerring, M., Underhaug, J., Miller, M., Frigaard, N.U., Nielsen, J.T. and Nielsen, N.C. (2012) In situ solid-state NMR spectroscopy of protein in heterogeneous membranes: the baseplate antenna complex of *Chlorobaculum tepidum*. Angew. Chem., Int. Ed. Engl. 51, 6891–6895.
[54] Jacso, T., Franks, W.T., Rose, H., Fink, U., Broecker, J., Keller, S., Oschkinat, H. and Reif, B. (2012) Characterization of membrane proteins in isolated native cellular membranes by dynamic nuclear polarization solid-state NMR spectroscopy without purification and reconstitution. Angew. Chem., Int. Ed. Engl. 51, 432–435.
[55] Yang, J., Tasayco, M.L. and Polenova, T. (2009) Dynamics of reassembled thioredoxin studied by magic angle spinning NMR: snapshots from different time scales. J. Am. Chem. Soc. 131, 13690–13702.